USSN: 09/616,283; Art Unit: 1645
Attorney Docket No. VRXB-P01-001

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

GOODNOW

Serial No: 09/616,283

Filed: July 14, 2000

For: SYSTEM FOR DETECTING BACTERIA IN BLOOD, BLOOD PRODUCTS, AND FLUIDS OF TISSUES

Art Unit: 1645

Attorney Docket No. VRXB-P01-001

Examiner: J. Hines

Assistant Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Declaration Under 35 U.S.C. §1.132

I, Jeffrey A. Hall, Ph.D., of Franklin, MA, hereby declare as follows:

1. I am the Director of Assay Development at Verax Biomedical, Inc., the assignee of the present application. I have been conducting research in immunoassay development for 13 years. Accordingly, my curriculum vitae is attached as Appendix A.

2. I have read the above-identified application, the pending claims, the Office Action mailed on February 11, 2003 and the Office action mailed on September 29, 2003.

3. I understand that the Examiner has stated that the invention as described and claimed in the above-identified application is obvious in view of the teachings of Fisher et al. WO 98/57994, McLaughlin (U.S. Patent 4,683,196), Tadler et al. (*J. Clin. Lab. Anal.* 3: 21-25 (1989)), Erich et al. (*J. Immunol.* 143(12): 4053-4060, 1989), and Chang et al. (U.S. Patent 5,200,323).

4. The Examiner states that McLaughlin teaches antibodies which specifically bind to gram negative bacteria in order to determine their presence and/or absence while Tadler et al., teach well known binding agents that bind lipotechoic acid of gram-positive bacteria in assays. See Office Action dated February 11, 2003. In the current Office Action the Examiner admits that the McLaughlin antibodies are not pan-generic but states that it would have been to obvious modify the assay to include the antibodies taught by Erich et al. instead of the McLaughlin antibodies. Similarly, Examiner admits that the Tadler et al. antibodies are not pan-generic but states that it would have been obvious to modify the assay to include the antibodies taught by Fischer et al. instead of the Tadler et al. antibodies.

5. I have reviewed the disclosures of all the cited references: McLaughlin, Erich et al., Tadler et al., and Fischer et al. For the reasons set forth below and the accompanying experimental data, I believe that the prior art antibodies fail to demonstrate broad pan-generic cross-reactivity and detection at a level of sensitivity to be effective in detecting clinically relevant amounts of bacteria in a blood or blood products as required by the claims.

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6. Verax Biomedical, Inc., has developed pan-generic antibodies immunoreactive with the Gram-negative antigen lipopolysaccharide (LPS) and pan-generic antibodies immunoreactive with the Gram-positive antigen lipoteichoic acid (LTA). The pan-generic activity and sensitivity of these antibodies has been compared with the closest commercially available antibodies that are being marketed for pan-generic reactivity. We

set forth below the comparative results obtained for the Verax gram positive as well as the gram negative antibodies.

Gram-Positive Antibodies

7. The Examiner states that Tadler et al disclose well known binding agents that bind the lipoteichoic acid (LTA) of the Gram-positive bacteria. We purchased commercially available antibodies that bind the LTA of the Gram-positive bacteria [Appendix B] and performed a side-by-side comparison with the Verax antibodies (VERAX PGD BA-3).[1]

8. The specification as filed discloses how to make and use pan-generic gram positive antibodies. Example 9 of the application demonstrates to one of ordinary skill in the art that the Verax monoclonal antibody clone 96-110 (described in WO 98/57994 by Fisher et al.; now designated as Verax PGD BA-3) shows pan-generic reactivity with seven Gram-positive bacteria as depicted in Figure 5 of the application. [Appendix C].

9. In addition, to the examples described in Example 9, we have generated and screened for additional antibodies that are pan-generic in nature and are capable of detecting clinically relevant amounts of bacteria using the methods taught in the specification at pages 24-26. VERAX PGD BA-4 is an example of such an antibody.

10. We conducted further assays comparing the Verax antibodies, such as VERAX PGD BA-3 and VERAX PGD BA-4 to commercially available antibodies from HyCult Biotech and Biogenesis, Inc. We evaluated the ability of these antibodies to detect clinically

[1] The VERAX PGD BA-3 antibody is the same as the antibody described in Example 9, i.e., the Fischer antibody of clone 96-110.

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relevant amounts of bacteria and to be useful in constructing meaningful screening assays. These results are presented below:

PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM POSITIVE BACTERIA

Vendor	Antigen	TEST BACTERIA: Staph epidermidis	Staphylococcus aureus	Staph lugdunensis	Bacillus subtilis	Group B Streptococcus	Group G Streptococcus	Enterococcus faecalis	Corynebacterium sps	Clostridium perfringes
HyCult Biotech	G+ LTA	28.1	1.2	1.4	1.3	1.5	3.8	1.9	3.8	1.7
Biogenesis Inc.	G+ LTA	9.2	16.3	1.1	2.9	1.5	6.7	1.3	6.7	2.2
VERAX PGD BA-3	G+ LTA	62.6	7.6	12.5	20.3	5.6	14.8	5.8	14.8	4.7
VERAX PGD BA-4	G+ LTA	77.9	30.1	71.3	10.2	5.6	10.8	21.4	10.7	2.8

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS. A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

Our results show that the commercially available antibodies were not truly pan-generic with respect to the detection of the LTA on Gram-positive bacterium and would not be useful in constructing a meaningful blood screening assay. In contrast, the Verax antibodies detected seven genera of Gram-positive bacterium routinely found in contaminated blood. See results above and Figure 5.

11. To be effective, a signal: noise ratio greater than two (2) is required for constructing a meaningful assay. The commercially available antibodies failed to demonstrate a ratio greater than two. As can be seen from the Table above, the Hycult Biotech antibody

failed to detect two species of *Staphylococcus* bacterium, *Bacilis subtilis*, Group B *Streptococcus*, *Enterococcus*, *Corynebacterium*, and *Clostridium*. Similarly, the Biogenesis Inc. antibody failed to detect one species of *Staphylococcus* bacterium, Group B *Streptococcus and Enterococcus*. In contrast, the Verax antibodies were effective in detecting seven different genera of bacteria and the signal was considerably stronger than that observed for the commercially available antibodies.

12. We now provide a side-by-side comparison of the pan-generic reactivity of the Verax antibodies to the Tadler et al. antibodies. Tadler et al. discloses an immunoassay for the detection of the LTA on gram positive bacteria. A close review of their experimental data shows that their antibodies demonstrate limited binding and detection of four bacterial genera: *Streptococcus spp.*, *Staphylococcus spp.*, *Enterococcal spp.*, and *Clostridium*. In contrast, the Verax antibodies are capable of pan-generic binding and detection of at least seven Gram-positive bacterial genera.

13. We further provide a comparison of the sensitivity of the Verax antibodies to the Tadler et al. antibodies. Figure 2 of Tadler et al. shows that only 2 bacterial genera, i.e., *Streptococcus mutans* and *Staphylococcus epidermidis*, are detected at clinically relevant amounts, $5x10^5$ CFU/50µl (i.e., $1x10^7$ CFU/ml). Thus, at this level of sensitivity the Tadler antibodies are not truly pan-generic. The bacteria *Staphylococcus aureus* is detected at $5x10^6$ CFU/50µl (i.e., $1x10^8$ CFU/ml), a level that is not clinically relevant. Additionally, the Tadler et al. immunoassay was unable to detect *Staphylococcus faecuim* at $5x10^6$ CFU/50µl ($1x10^8$ CFU/ml) suggesting that the antibodies are only able to cross-react with LTA on certain Staphylococcus *spp.* and Streptococcus *spp.* Therefore, in

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effect, Tadler et al. show detection of only two genera of gram-positive bacteria at clinically relevant amounts as is required by the claims. See Figure 2 of Tadler et al.

14. In contrast, the following Table set forth both the pan-generic cross-reactivity of the Verax antibodies, as well as the sensitivity of these antibodies, demonstrating a greater degree of sensitivity in detecting clinically relevant amounts of bacteria in contaminated blood or blood products ($1x10^2$ CFU/ml – $1x10^6$ CFU/ml).

GRAM POSITIVE RAPID TEST SIGNAL (G/DENS)

CFU/ml	S. epidermidis	S. aureus	S. lugdenensis	B. cereus	GRP B Strep	GRP G Strep	S. pyogenes	E. faecalis	C. minutissimum	C. perfringes	CFU/ml
1.0 E5	17.26	1.04	13.21	12.24	0.26	16.07	nt	13.85	6.82	11.20	1.0 E5
5.0 E4	nt	nt	3.20	9.91	nt	nt	21.77	nt	nt	nt	5.0 E4
1.0 E4	8.81	0.41	0.38	2.45	0.04	1.43	10.32	3.19	2.03	8.67	1.0 E4
5.0 E3	3.98	nt	nt	nt	0.04	nt	nt	0.44	nt	nt	5.0 E3
1.0 E3	0.83	0.24	0.17	0.29	nt	0.46	0.51	0.27	1.26	2.91	1.0 E3

*BOXED CELL = MINIMALL DETECTABLE CONCENTRATION
**G/DENS = REFLECTANCE SIGNAL, ANY G/DENS > 0.25 IS VISIBLE
***nt = NOT TESTED

15. We now examine the pan-generic cross-reactivity of the Fischer et al antibody as taught by the Fischer PCT publication . The Fischer PCT publication referenced by the Examiner fails to disclose or suggest pan-generic activity. The Table below provides a comparison of the properties shown in the Fischer references in comparison to the properties of the Fischer antibody now appreciated and recognized by Verax.

Gram positive bacterial species recognized by the Fisher antibody as taught by the Fischer reference	Gram positive bacterial species recognized by the Fisher antibody as taught by the instant application

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Staphylococcus spp.	*Staphylococcus spp.* *Streptococcus pyogenes* *Group B Streptococcus* *Group G Streptococcus* *Enterococcus faecalis* *Corynebacterium minutissimum* *Clostridium perfringens* *Bacillus spp.*

Thus, the Fisher et al. reference shows binding to ***only*** one bacterial genus: the Staphylococcus genus: there was no recognition of its pan-generic ability in the Fischer reference.

Gram-Negative Antibodies

16. The Examiner states that McLaughlin and/or Erich et al. disclose antibodies which specifically bind to gram-negative bacteria. McLaughlin discloses mouse and rabbit antibodies that bind to the Lipid A core of the LPS on Gram-negative bacteria. Erich et al disclose three murine monoclonal antibodies and state that one of the three antibodies showed cross-reactivity with the heterlogous LPS and Gram bacterial strains but cross-reactivity was either moderate or virtually absent for the remaining two. See Page 4058. We conducted a side-by-side comparison of the Verax antibodies to the closest commercially available mouse antibodies that are marketed as being anti-LPS core or anti-endotoxin [Appendix D] from HyCult Biotech, Virostat, and QED.

17. The specification as filed discloses how to make and use pan-generic gram negative antibodies. Example 9 of the application demonstrates to one of ordinary skill in the art

that the Verax monoclonal antibody clone 26-5 (commercially available from Biodesign International) shows pan-generic reactivity with the LPS of seven Gram-negative bacteria as depicted in Figure 6 of the application. [Appendix E].

18. In addition to the examples described in Example 9, we have generated and screened for additional antibodies that are pan-generic in nature and are capable of detecting clinically relevant amounts of bacteria using the methods taught in the specification at pages 24-26. VERAX PGD BA-1 and VERAX PGD BA-1 are examples of such antibodies.

19. McLaughlin antibodies are reactive with gram-negative bacteria such as *Neisseria, Chlamydia, and Salmonella*. The Erich et al antibodies are cross-reactive with only two (live) bacterial genera: *Escherichia* and *Salmonella*. In contrast, the Verax antibodies show pan-generic activity against a range of bacteria that have been identified as contaminants in blood and blood products in three major national transfusion reaction studies including the BaCon Study in the United States, the Hemovigilance study in France, and the SHOT study in the United Kingdom. These contaminants include both pathogenic as well as non-pathogenic bacterial species such as *Yersinia entercolitica* and *Proteous miribilis*, along with other common soil-borne bugs. The McLaughlin and Erich et al antibodies appear to be effective mainly against the pathogenic gram negative bacterial species (e.g., *Neisseria, Escherichia,* and *Chlamydia*).

20. In addition, we conducted further assays using Verax antibodies, in evaluating the ability of these antibodies to detect clinically relevant amounts of bacteria and be useful in constructing meaningful screening assays. These results are presented below:

USSN: 09/616,283; Art Unit: 1645
Attorney Docket No. VRXB-P01-001

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PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM NEGATIVE BACTERIA

TEST BACTERIA

Vendor	Antigen	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Acinetobacter baumanii*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella enteritidis*	*Yersinia enterocolitica*	*Proteus mirabilis*	*Serratia marcescens*
HyCult Biotech	LPS	1	1	1.1	1.2	1.1	1.2	1	1	1	1.3	1.3
Virostat	LPS	1	1	1	1	1	1	1	1	1	1	1.1
QED	LPS	1	1	1	1	1	1	NT	NT	NT	NT	NT
VERAX PGD BA-1	LPS	12.1	11.1	12.5	12.8	12.3	9.6	9.7	11.2	11.4	10.6	12.3
VERAX PGD BA-2	LPS	11.2	18.5	14.2	9.9	7.8	13.5	9.2	8.5	12.1	16.5	22.7

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

21. To be effective, a signal: noise ratio greater than two (2) is required for constructing a meaningful assay. As can be seen from the Table above, the commercially available antibodies failed to detect all nine bacterial genera. In contrast, the Verax antibodies were effective in detecting nine different genera of bacteria and showed strong signals for each of these bacteria. Thus, the Verax antibodies showed greater effectiveness than the commercially available antibodies.

22. In addition, the following Table sets forth the sensitivity of the Verax gram negative antibodies, demonstrating a greater degree of sensitivity in detecting clinically relevant amounts of bacteria in contaminated blood or blood products ($1x10^2$ CFU/ml – $1x10^6$ CFU/ml).

USSN: 09/616,283; Art Unit: 1645
Attorney Docket No. VRXB-P01-001

VERAX PLATELET PGD ASSAY: ANALYTICAL SENSITIVITY

	GRAM NEGATIVE RAPID TEST SIGNAL (G/DENS)										
CFU/ml	*E. coli*	*Ps. aeruginosa*	*E. aerogenes*	*K. oxytoca*	*E. cloacae*	*A. baumanii*	*K. pneumoniae*	*S. enteritidis*	*P. mirabilis*	*S. marcescens*	CFU/ml
1.0 E5	4.51	2.16	9.13	8.68	1.01	7.71	5.30	1.14	1.38	0.83	1.0 E5
5.0 E4	4.10	1.45	7.70	7.08	0.77	7.51	4.40	0.70	0.98	0.71	5.0 E4
1.0 E4	2.75	0.79	6.88	3.31	0.82	4.79	2.34	0.50	0.56	0.69	1.0 E4
5.0 E3	1.34	0.67	4.81	1.46	0.73	2.98	1.21	0.48	0.24	0.16	5.0 E3
1.0 E3	0.99	0.32	4.10	0.25	0.75	1.46	0.57	0.04	0.09	0.01	1.0 E3

23. For these reasons, I believe that the closest commercially available prior art antibodies do not show the pan-generic activity or sensitivity required to be effective in detecting clinically relevant amounts of bacterial contaminants in blood and blood products.

24. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated:_____ Signature: _____

Dr. Jeffrey A. Hall, Ph.D
Director, Assay Development
Verax Biomedical Incorporated